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(54) Title: NOVEL CONJUGATES OF OPIOIDS AND ENDOGENOUS CARRIERS		
(57) Abstract Conjugates are prepared from antinociceptive agents, particularly opioids or opioid analogs, more particularly dynorphins, endorphins, deltorphins, enkephalins or analogs thereof, by combining said antinociceptive agent with a material providing a functionally reactive group capable of reacting with a blood component (preferably a blood cell or protein). Said conjugates permit extension of the therapeutic life of the antinociceptive agent. They may be administered to patients to alleviate pain, produce analgesic effects, or assist in cases of narcotics withdrawal, and may also be used as probes for receptor activity. The administration to the patient may be made either <i>in vivo</i> or <i>ex vivo</i> and may be performed by either introducing the derivative including the reactive functional group into the patient's vascular system or preparing such a conjugate externally (or <i>in vitro</i>) and introducing that conjugate to the patient's vascular system.		

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NOVEL CONJUGATES OF OPIOIDS AND ENDOGENOUS CARRIERS

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FIELD OF THE INVENTION

This invention relates to conjugates of antinociceptive agents, notably opioids, and endogenous carriers, particularly to opioids and various blood components, particularly blood proteins.

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BACKGROUND OF THE INVENTION

Antinociceptive agents comprise a large class of drugs that are used to alleviate pain. They include compounds such as steroids, analgesics, barbiturates and opioids.

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The opioids comprise a large class of drugs, clinically used to relieve pain, and which include both plant-derived and synthetic alkaloids and peptides found indigenously in brains of mammals. The latter comprise three distinct families: beta-endorphin and other peptides derived from proopiomelanocortin, the enkephalins and the dynorphins. Opioids interact with neuronal cells and modulate physiological functions such as nociception. One of the physiological effects attributed to this class of compounds is analgesia.

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While opioid drugs are used clinically to relieve pain their usefulness is limited by the tolerance and dependence that normally develops on chronic treatment. Opioid drugs such as morphine can be addictive and can have central mediated side effects such as respiratory and cardiac depressions and drowsiness. It would be desirable to develop therapeutic agents that could utilize the pain alleviating properties of the opioids without, or with lessened, central

mediated side effects. It would also be desirable to be able to develop therapeutic agents which retain the positive properties of opioids and/or other antinociceptive agents for longer periods of time than is normally currently the case.

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SUMMARY OF THE INVENTION

This invention relates to novel chemical reactive derivatives of antinociceptive agents, particularly opioids, which can react with available reactive functionalities on blood components to form covalent linkages, and in which the resulting covalently bound conjugates have antinociceptive activity.

As compared with the parent drugs the conjugated molecules have extended lifetimes in the bloodstream and are, therefore, capable of maintaining activity for extended periods of time as compared to the unconjugated parent drug, and of providing such activity with minimal or no centrally mediated side effects.

The invention also includes the conjugates of these drugs with blood components and methods for providing activity to a patient comprising administering to the bloodstream of a mammalian host the novel antinociceptive agent derivatives or the novel conjugates.

This invention relates to the use of the derivatives of this invention for the treatment of pain as well as to modify the immune response in patients.

This invention also relates to use of antibodies to locate and bind to such conjugates, for instance, to remove undesirable excesses of them from the host's blood stream.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is dynorphin A (1-17).
SEQ ID NO:2 is a dynorphin analogue, Dyn A (1-13).
SEQ ID NO:3 is a dynorphin analogue, Dyn A (2-13).
SEQ ID NO:4 is a derivative of Dyn A (1-13).

SEQ ID NO:5 is another derivative of Dyn A (1-13).

SEQ ID NO:6 is a derivative of Dyn A (2-13).

SEQ ID NO:7 is a derivative of Dyn A (2-17).

5 **DETAILED DESCRIPTION OF THE INVENTION**

To ensure a complete understanding of the invention, the following definitions are provided:

10 **Antinociceptive agents:** Antinociceptive agents are drugs that are used to alleviate pain. Antinociceptive agents include steroids, analgesics, barbiturates and opioids.

15 **Opioids:** Opioids are a large class of drugs, used clinically as painkillers, that include both plant-derived and synthetic alkaloids and peptides found endogenously in the mammalian brain. While the plant-derived alkaloids have been known and used for thousands of years, the endogenous opioid peptides were discovered only in the mid-1970s.

20 Opioids include endorphins, enkephalins, deltorphins, dynorphins, and analogs and derivatives of these. Of the opioids the dynorphins, and particularly dynorphin A and its derivatives and analogs, are preferred for use in this invention.

25 **Dynorphins:** Dynorphins are a class of endogenous opioids that exist in multiple forms in the central nervous system. Dynorphins are derived from the precursor prodynorphin (proenkephalin B). Dynorphin, also known as Dynorphin A1-17, is a well-known opioid that has the sequence Tyr-Gly-Gly-Phe-Leu⁵-Arg-Arg-Ile-Arg-Pro¹⁰-Lys-Leu-Lys-Trp-Asp¹⁵-Asn-Gln. SEQ ID NO:1. A
30 number of derivatives and analogs of dynorphin are known including Dyn A1-13, SEQ ID NO: 2 Dyn A2-13, SEQ ID NO:3, Dyn A1-12, Dyn A2-12 and Dyn A2-17 as well as amide analogs such as those

mentioned in U.S. Patent 4,462,941 of Lee et al., N-terminus truncated dynorphin analogs such as those described in International Patent Application WO 96/06626 of Lee et al. and des-Tyr or des-Tyr-Gly analogs such as those disclosed in International Patent Application WO 93/25217 also of Lee et al.

Opioid Receptors: Opioid receptors are membrane bound receptors to which opioid molecules bind. Morphine binds to μ opioid receptors. Enkephalins bind to δ opioid receptors. Dynorphin peptides bind to κ opioid receptors.

Receptor Agonists: Receptor agonists are chemical substances capable of activating a receptor to induce a full or partial pharmacological response.

Receptor Antagonists: Receptor Antagonists are chemical substances that are structurally related to a biologically active substance and which acts as an inhibitor.

Reactive Entities: Reactive entities are entities capable of forming a covalent bond. Such reactive agents are coupled or bonded to a therapeutic or diagnostic agent of interest. Reactive entities will generally be stable in an aqueous environment and will usually be a carboxyl, phosphoryl, or convenient acyl group, either as an ester or a mixed anhydride, or an imidate, thereby capable of forming a covalent bond with a group at the target site to form a derivative.

The reactive functionalities available on vascular proteins for covalent bond formation with the reactive group are primarily amino, carboxyl, hydroxyl and thiol groups.

Taking into account these definitions, this invention relates to compositions which are derivatives of antinociceptive agents,

preferably of opioids, most preferably of dynorphins or a dynorphin derivative or analog, which can react with the available reactive functionalities on blood components via covalent linkages. The invention also relates to such derivatives, such combinations with
5 blood components, and methods for their use. These methods include methods that extend the effect of therapeutic life of the drug in question as compared to administration of the parent drug per se to a patient, and methods for alleviating pain.

The derivative is of a type designated as a DAC (Drug Affinity
10 Complex) which comprises the antinociceptive agent molecule and a linking group together with a chemically reactive group as described herein capable of reaction with a reactive functionality of a blood component, particularly of a blood protein. The blood protein may be blood derived, purified from blood or a recombinant blood protein.
15 By reaction with the blood component or protein the derivative or DAC may be delivered via the blood to appropriate sites or receptors of the patient.

Derivatives of opioids and other antinociceptive agents which can conjugate with proteins and other blood components are
20 prepared as is known in the art for other therapeutic drugs, e.g. as in U.S. Patent 5,612,034, by the use of linking groups having chemically reactive groups which covalently bond to reactive functionalities on proteins, as described above. These reactive functionalities are primarily amino, carboxyl, hydroxyl and thiol
25 groups. To form covalent bonds with the functional group on the protein, one may use as a chemically reactive group a variety of active groups. While a number of different reactive groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimidyl (NHS) and maleimido. The introduction of these
30 groups may be accomplished, for example, by the use of *N*-hydroxysuccinimide (NHS), *N*-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimide-

butyrylsuccinimide (GMBS), maleimidopropionic acid (MPA) or any agent affording an NHS ester or maleimido group. In the preferred embodiments of this invention, the functional group on this protein will be a thiol group and the chemically reactive group will be a maleimido-containing group.

Other linking agents that may be utilized are described in U.S. Patent 5,612,034, which is hereby incorporated herein.

The antinociceptive agents which may be utilized in this invention are those which can be combined with such linking groups and chemically reactive groups, similarly to the opioids, so as to be capable of forming covalent bonds with functional groups on proteins.

To the extent that targeted bonding is employed, the choice of the long-lived blood component will be affected, at least in part, by the desired lifetime for the drug and the availability of the blood component for bonding to the opioid derivative. The various sites with which the chemically reactive group of the subject opioid derivatives may react include cells, particularly red blood cells (erythrocytes) and platelets, proteins, such as immunoglobulins, including IgG and IgM, serum albumin, ferritin, steroid binding proteins, transferrin, thyroxin binding protein, α -2-macroglobulin, and the like. Those proteins with which the derivatized opioids react, which are not long-lived, will generally be eliminated from the host within about three days. The proteins indicated above (including the proteins of the cells) will remain at least three days, and may remain five days or more (usually not exceeding 60 days, more usually not exceeding 30 days) particularly as to the half life, based on the concentration in the blood.

For the most part, reaction will be with mobile components in the blood, particularly blood proteins and cells, more particularly blood proteins and erythrocytes. By "mobile" is intended that the component does not have a fixed situs for any extended period of

time, generally not exceeding 5, more usually one minute, although some of the blood component may be relatively stationary for extended periods of time. Initially, there will be a relatively heterogeneous population of functionalized proteins and cells.

5 However, for the most part, the population within a few days will vary substantially from the initial population, depending upon the half-life of the functionalized proteins in the blood stream. Therefore, usually within about three days or more, IgG will become the predominant functionalized protein in the blood stream.

10 Usually, by day 5 post-administration, IgG, serum albumin and erythrocytes will be at least about 60 mole %, usually at least about 75 mole %, of the conjugated components in blood, with IgG, IgM (to a substantially lesser extent) and serum albumin being at least about 50 mole %, usually at least about 75 mole %, more usually at
15 least about 80 mole %, of the non-cellular conjugated components.

Preferably, the antinociceptive agent or opioid derivative is conjugated to albumin. Such conjugation is preferably established by covalent bonding of a maleimide (e.g., prepared from GMBS, MPA or another maleimido group) to a thiol group on the albumin. As there
20 is only a single thiol group on albumin, conjugates will tend to comprise approximately a 1:1 ratio of opioid derivatives to albumin. This is in opposition to typical conjugation techniques that result in multiple copies of the therapeutic drug in question being covalently bound to a single albumin molecule.

25 If desired, the subject conjugates may also be prepared *ex vivo* by combining blood with derivatized opioids or other agents of the present invention, allowing covalent bonding of the derivatized drugs to reactive functionalities on blood components and then returning or administering the conjugated blood to the host. Moreover, the above
30 may also be accomplished by first purifying an individual blood component or limited number of components, such as red blood cells, immunoglobulins, serum albumin, or the like, and combining

the component or components *ex vivo* with the chemically reactive derivatives. The functionalized blood or blood component may then be returned to the host to provide *in vivo* the subject therapeutically effective conjugates. The blood also may be treated to prevent
5 coagulation during handling *ex vivo*.

Some blood components such as hemoglobin are known to possess comparatively high permeability across the blood-nerve and blood-brain barriers. One focus of a preferred embodiment of this invention utilizes the ability of albumin to enter the interstitial space
10 and gain access to peripheral neurons so as to deliver modified opioid molecules to pain receptors to influence pain transmission via the stimulation of peripheral opioid receptors. Clinical data has suggested that peripheral opioid receptors can be a potential target for relevant antinociceptive activity of morphine-like drugs and be
15 effective in limiting pain without the need for penetration into the central nervous system (Stein et al., 1991). The major limitations of existing opioid-like drugs include central mediated side effects (respiratory and cardiac depressions), addictive potential and down regulation or loss of efficacy. In contrast drugs bound to plasma
20 proteins such as albumin would retain activity and be devoid of the central mediated side effects such as cardiac and respiratory depression and addiction. Preferably the conjugates of this invention are constructed so as to selectively react and covalently bond with thiol groups on proteins, most preferably with proteins which do not
25 cross the blood-brain or blood-nerve barriers. Such conjugates can deliver the antinociceptive effect of the drug without effect on the brain or on the central nervous system. However, should it be desirable to produce conjugates that can cross these barriers, then the antinociceptive agent is derivatized with a more generally
30 reactive group such as a succinimide. Such derivatives can react with various blood proteins and other components non-selectively, so that the possible conjugates include those that can cross the barrier.

Therefore, in a preferred embodiment of this invention, to aid in minimizing centrally mediated side effects, in addition to constructing the derivatives so as to conjugate primarily with albumins, the ratio of antinociceptive derivatives to blood is controlled so as to take advantage of this comparatively large amount of albumin (the preferred blood component for forming conjugates) in the blood. Preferably, the amount of antinociceptive derivatives added to blood in vivo or ex vivo is from about 0.01 $\mu\text{mol/kg}$ to about 100 $\mu\text{mol/kg}$, most preferably from about 1 $\mu\text{mol/kg}$ to about 30 $\mu\text{mol/kg}$.

Thus, the derivative of the antinociceptive agent (or opioid) may be designed either for random (nonselective) or targeted (selective) bonding, with blood components in general, or with selected components (such as albumin). Targeted or selective bonding may be accomplished, as described above, by incorporating into the derivative a reactive group that will selectively bond to a desired blood component. Alternatively one may prepare a combinatorial library and screen for members of that library which provide the desired blood component association spectrum.

A conjugate of an opioid of this general type was prepared in Kieffer, et al., *Analytical Biochemistry* vol. 215 p. 1 (1993) from a peptide prepared by the authors (designated Peptide B in the reference), with bovine albumin (BSA) via a maleimide linker (MBS). Peptide B has the sequence YdAGFLTPRRASLGC, in which dA stands for d-alanine. The conjugate was determined to have better binding potency for the δ -opioid receptor than Peptide B itself. However, no mention is made of any therapeutic effect of this conjugate.

The desired conjugates of opioids or other antinociceptive drugs to blood components may be prepared *in vivo* by administration of the opioid or other derivative to the patient, which may be a human or other animal. The administration may be done in

the form of a bolus or introduced slowly over time by infusion using metered flow or the like. Alternatively, blood may be removed from the host, treated *ex vivo* and returned to the host. Another application requires *ex vivo* conjugation of the opioid or other derivative to a commercial source of plasma protein (e.g. albumin) followed by infusion to the host.

For in vivo or ex vivo conjugate formation, the drug derivatives will be administered in a physiologically acceptable medium, e.g. deionized water, phosphate buffered saline (PBS), saline, aqueous ethanol or other alcohol, plasma, proteinaceous solutions, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. If necessary a small amount of a physiologically acceptable solvent or co-solvent such as DMSO may be included. Other additives which may be included include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM, salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, and the like. The compositions may be lyophilized for convenient storage and transport.

The subject drug derivatives will for the most part be administered parenterally, such as intravascularly, (IV), intraocular (IO), intraarterially (IA), intramuscularly (IM), subcutaneously (SC), or the like. Administration may in appropriate situations be by transfusion. In some instances, where reaction of the active functional group is relatively slow, administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the conjugate allows for transfer to the vascular system. Usually a single injection will be employed although more than one injection may be used, if desired. The drug derivatives may be administered by any convenient means, including syringe, trocar, catheter, or the like. The particular manner of administration will vary depending upon the amount to be administered, whether a single bolus or continuous

administration, or the like. Preferably, the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. Other routes may find use
5 where the administration is coupled with slow release techniques or a protective matrix. The intent is that the antinociceptive agent, particularly the opioid, dynorphin analog, or derivative be effectively distributed in the blood, so as to be able to react with the blood components. The concentration of the conjugate will vary widely,
10 generally ranging from about 1 pg/ml to 50 mg/ml. The total administered intravascularly will generally be in the range of about 0.1 mg/ml to about 10 mg/ml, more usually about 1 mg/ml to about 5 mg/ml.

By bonding to long-lived components of the blood, such as
15 immunoglobulin, serum albumin, red blood cells and platelets, a number of advantages ensue. The activity of the drug is extended for days to weeks. Only one administration need be given during this period of time. Greater specificity can be achieved, since the active compound will be primarily bound to large molecules, where it
20 is less likely to be taken up intracellularly to interfere with other physiological processes.

The blood of the mammalian host may be monitored for the presence of the drug one or more times. By taking a portion or sample of the blood of the host, one may determine whether the
25 drug has become bound to the long-lived blood components in sufficient amount to be therapeutically active and, thereafter, the level of that compound in the blood. If desired, one may also determine to which of the blood components the drug or its derivative molecule is bound.

30 Thus, this invention relates to such conjugates of antinociceptive agents, particularly opioids, opioid analogs and their derivatives with blood components, particularly blood proteins such

as albumin, as well as methods of administering them to human and other animal patients.

Another aspect of this invention relates to methods for determining the concentration of the drug, or its derivatives and conjugates in biological samples (such as blood) using antibodies specific to the antinociceptive agent or its derivatives and conjugates, and to the use of such antibodies as a treatment for toxicity potentially associated with such drugs or conjugates. This is advantageous because the increased stability and life of the drugs *in vivo* in the patient might lead to novel problems during treatment, including increased possibility for toxicity. The use of anti-therapeutic agent antibodies, either monoclonal or polyclonal, having specificity for a particular antinociceptive agent or derivative thereof, can assist in mediating any such problem. The antibody may be generated or derived from a host immunized with the particular drug or derivative thereof, or with an immunogenic fragment of the agent, or a synthesized immunogen corresponding to an antigenic determinant of the agent. Preferred antibodies will have high specificity and affinity for native, derivatized and conjugated forms of the antinociceptive agent. Such antibodies can also be labeled with enzymes, fluorochromes, or radio labels.

The anti-therapeutic agent antibodies may be used to treat toxicity induced by administration of the antinociceptive agent or derivative thereof, and may be used *ex vivo* or *in vivo*. *Ex vivo* methods would include immuno-dialysis treatment for toxicity employing anti-therapeutic agent antibodies fixed to solid supports. *In vivo* methods include administration of anti-therapeutic agent antibodies in amounts effective to induce clearance of antibody-agent complexes.

The antibodies may be used to remove the antinociceptive agent, and conjugates thereof, from a patient's blood *ex vivo* by contacting the blood with the antibodies under sterile conditions.

For example, the antibodies can be fixed or otherwise immobilized on a column matrix and the patient's blood can be removed from the patient and passed over the matrix. The antinociceptive agent or conjugates will bind to the antibodies and the blood containing a low concentration of the antinociceptive agent or conjugate, then may be returned to the patient's circulatory system. Adjusting the pressure and flow rate can control the amount of antinociceptive agent removed. Preferential removal of the antinociceptive agent and conjugates from the serum component of a patient's blood can be effected, for example, by the use of a semipermeable membrane, or by otherwise first separating the serum component from the cellular component by ways known in the art prior to passing the serum component over a matrix containing the anti-therapeutic antibodies. Alternatively the preferential removal of conjugated blood cells, including red blood cells, can be effected by collecting and concentrating the blood cells in the patient's blood and contacting those cells with fixed anti-therapeutic antibodies to the exclusion of the serum component of the patient's blood.

The anti-therapeutic antibodies can be administered *in vivo*, parenterally, to a patient that has received the antinociceptive agents or conjugates for treatment. The antibodies will bind the compounds and conjugates. Once bound the drug's activity will be hindered if not completely blocked thereby reducing the biologically effective concentration of antinociceptive agents in the patient's bloodstream and minimizing harmful side effects. In addition, the bound antibody-drug complex will facilitate clearance of the antinociceptive agents and conjugates from the patient's blood stream.

The derivatives and conjugates of the antinociceptive agents may be used in several different ways and to achieve several different ends. As mentioned above, these materials may be used in place of typical antinociceptive agents for alleviating pain. As compared with drugs currently available, the materials of this

invention can alleviate pain without central mediated side effects or potential of addiction or loss of efficacy, and are available for alleviating pain for a substantially longer time than conventionally administered drugs. Opioid derivatives and conjugates of this invention also may be utilized (in accordance with U.S. Patent 5,482,930) as anti-inflammatory and/or anti-irritation agents or in general to inhibit vascular leakage from tissues. In addition, as is known in the art, these materials may be used to treat hosts which are or have become tolerant to morphine (or to treat patients undergoing methadone treatment programs), as well as treatment of narcotics withdrawal in general. The conjugates and materials of this invention in addition, when labeled, may be utilized for experimental purposes such as probes to investigate biological functions of various receptors.

The invention is further illustrated by the following examples.

Experimental Section

General

Syntheses of all dynorphin A analogs were performed using manual solid-phase synthesis and an ABI 433A Peptide Synthesizer using 0.55 mmol/g of Fmoc protected Rink Amide MBHA resin (NovaBiochem), 4 eq. of Fmoc protected amino acids, 4 eq of a 0.45 M O-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) in *N,N*-dimethylformamide solution as activation with 4 eq. of 2 M *N,N*-diisopropylethylamine (DIEA) in 1-methyl-2-pyrrolidinone (NMP), and piperidine deprotection of Fmoc groups. Side chain derivatization of the carboxy-terminal lysine residue was accomplished using Fmoc-Lys(Mtt)-OH (NovaBiochem) and deprotection of the methyltrityl (Mtt) group was accomplished with 5% trifluoroacetic acid (TFA)/5% triisopropylsilane (TIS) in dichloromethane (DCM). Derivatives with free amino-terminal amino

acid residues were synthesized using either Boc-Tyr(*t*Bu)-OH (NovaBiochem) or Boc-Gly-OH (Advanced Chem Tech). Resin cleavage and product isolation were all performed using 95% TFA/2.5% TIS/2.5% H₂O, followed by dry-ice cold Et₂O precipitation. All dynorphin A analogs were purified by preparative reversed phased HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 5-60% B (0.045% TFA in H₂O (A) and 0.045% TFA in CH₃CN (B)) at 9.5 mL/min using a Dynamax C₁₈, 60Å, 8 μm, 21 mm x 25 cm column equipped with a Dynamax C₁₈, 60Å, 8 μm guard module and an UV detector (Varian Dynamax UVD II) at 214 and 254 nm. Analytical HPLC were performed using a Varian (Rainin) binary HPLC system: gradient elution of 5-60% B (0.045% TFA in H₂O (A) and 0.045% TFA in CH₃CN (B)) at 0.5 mL/min using a Dynamax C₁₈, 60Å, 8 μm, 4.6 mm x 25 cm column equipped with a Dynamax C₁₈, 60Å, 8 μm guard module and an UV detector (Varian Dynamax UVD II) at 214 and 254 nm. Mass spectrometry was performed on a PE Sciex API III electro-spray Biomolecular Mass Analyzer.

Note that TFA would not be acceptable for inclusion in a product intended for use in humans, so that a human-compatible protective agent such as HCl would be used.

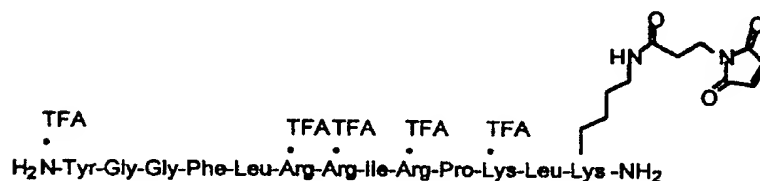
Example 1

Synthesis of Dyn A 1-13(MPA)-NH₂

Using automated peptide synthesis, the following protected amino acids were sequentially added to Ring Amide MBHA resin: Fmoc-Lys(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, and Boc-Tyr(Boc)-OH. Manual synthesis was employed for the remaining steps: selective removal of the Mtt group and coupling of maleimidopropionic acid (MPA) using HBTU/HOBt/DIEA activation in

DMF. The target dynorphin analog was removed from the resin; the product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization in a 42% yield. Anal. HPLC indicated product to be
 5 > 95% pure with $R_t = 33.00$ min. ESI-MS m/z for $C_{82}H_{133}N_{26}O_{17}$ (MH^+), calcd 1754.0, found 1754.4, MH^{3+} 585.8.

The structure of this product is



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Example 2

Synthesis of Dyn A 2-13(MPA)-NH₂

Using automated peptide synthesis, the following protected amino acids were sequentially added to Ring Amide MBHA resin:
 15 Fmoc-Lys(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, and Boc-Gly-OH. Manual synthesis was employed for the remaining steps: selective removal of the Mtt group and coupling of MPA using
 20 HBTU/HOBt/DIEA activation in DMF. The target dynorphin analog was removed from the resin; the product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization in a 35% yield. Anal. HPLC indicated product to be > 95% pure with $R_t = 30.42$ min.
 25 ESI-MS m/z for $C_{73}H_{123}N_{25}O_{15}$ (MH^+), calcd 1590.0, found MH^{3+} 531.3.

Example 3

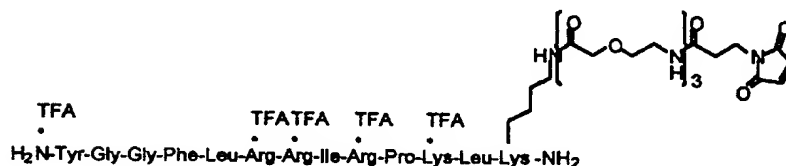
Synthesis of Dyn A 1-13(AEA₃-MPA)-NH₂

Using automated peptide synthesis, the following protected amino acids were sequentially added to Ring Amide MBHA resin:

- 5 Fmoc-Lys(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, and Boc-Tyr(Boc)-OH. Manual synthesis was employed for the remaining steps: selective removal of the Mtt group, the coupling of three-
 10 Fmoc-AEA-OH groups (AEA = aminoethoxyacetic acid) with Fmoc removal in-between each coupling, and MPA acid using HBTU/HOBt/DIEA activation in DMF. The target dynorphin analog was removed from the resin; the product was isolated by precipitation and purified by preparative HPLC to afford the desired
 15 product as a white solid upon lyophilization in a 29% yield. Anal. HPLC indicated product to be >95% pure with $R_t = 33.06$ min. ESI-MS m/z for C₉₄H₁₅₄N₂₉O₂₃ (MH⁺), calcd 2057.2, found MH⁴⁺ 515.4, MH³⁺ 686.9, MH²⁺ 1029.7.

The structure of this product is

20



Example 4

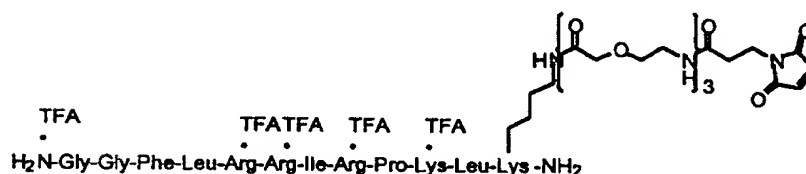
Synthesis of Dyn A 2-13(AEA₃-MPA)-NH₂

25

Using automated peptide synthesis, the following protected amino acids were sequentially added to Ring Amide MBHA resin: Fmoc-Lys(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-

OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, and Fmoc-Gly-OH. Manual synthesis was employed for the remaining steps: selective removal of the Mtt group, the coupling of three-Fmoc-AEA-OH groups, with Fmoc removal in-between each coupling, and MPA using HBTU/HOBt/DIEA activation in DMF. The target dynorphin analog was removed from the resin; the product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization in a 29% yield. Anal. HPLC indicated product to be >95% pure with $R_t = 31.88$ min. ESI-MS m/z for $C_{85}H_{145}N_{25}O_{21}$ (MH^+), calcd 1894.3, found MH^{4+} 474.6, MH^{3+} 632.4, MH^{2+} 948.10.

The structure of this product is

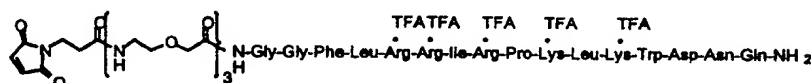


Example 5 Synthesis of MPA-AEA₃-Dyn A 2-17-NH₂

Using automated peptide synthesis, the following protected amino acids and maleimide were sequentially added to Ring Amide MBHA resin: Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-AEA-OH, Fmoc-AEA-OH, Fmoc-AEA-OH, and MPA. The target dynorphin analog was then removed from the resin; the product was isolated by precipitation and purified by preparative HPLC to afford the desired

product as a pale yellow solid upon lyophilization in a 32% yield. Anal. HPLC indicated product to be >95% pure with $R_t = 33.44$ min. ESI-MS m/z for $C_{109}H_{172}N_{35}O_{29}$ (MH^+), calcd 2436.8, found MH^{3+} 813.6.

5 The structure of this product is



Example 6

10 Preparation of Dynorphin-Human Serum Albumin Conjugates (*Ex Vivo* Preparation)

In 10 mL reaction vials were placed 4.95 mL of 20% HSA. To that was added 50.0 μ L of a 10 mM solution of the dynorphin derivative prepared in Example 1, 3, 4 or 5 dissolved in water.

15 These mixtures were allowed to stand at room temperature for 3 hours, then analyzed by reverse-phase HPLC, which showed the absence of the starting dynorphin derivative.

Example 7

20 Non-selective Binding Assay

Non-selective binding of the conjugates produced above was assayed using Naloxone as follows.

Binding Reaction

1. Each tube receives the following components:

- 25 25 μ L drug or vehicle
 25 μ L 3 H-Naloxone
 200 μ L tissue suspension (rat brain homogenate)

2. Initiate the binding reaction with the addition of tissue, and incubate for 90 minutes at 25 $^{\circ}$ C.

30 3. Terminate the binding reaction by rapid vacuum filtration of the

assay contents onto untreated GF/B filters.

4. Rinse the tubes once with ice-cold 50 mM TRIS•HCl (pH 7.4, at 25 °C), then rinse the filters with approximately 7 mL/tube of the same ice-cold wash buffer.
5. Radioactivity trapped on the filters is assessed using liquid scintillation spectrophotometry.

Materials and Reagents

1. [³H]-Naloxone is diluted in 50 mM Tris-HCl (pH 7.4 at 25° C) to a concentration of 10 nM, such that the final radioligand concentration in this assay is 1.0 nM.

2. Non-specific binding is defined as that remaining in the presence of 1 μ M Naloxone.

3. The reference compound is Naloxone at final concentrations of: 3×10^{-11} , 1×10^{-10} , 3×10^{-10} , 1×10^{-9} , 3×10^{-9} , 1×10^{-8} , 3×10^{-8} , 1×10^{-7} , and 3×10^{-7} M.

4. The positive control is Naloxone run at final concentrations of 3×10^{-9} , 3×10^{-8} and 3×10^{-7} M.

5. The K_d of the mu opiate receptor for [³H]-Naloxone is 2.0 nM.

- 20 The assay was conducted using HSA and the initial dynorphins (A 1-13, A 2-13 and A 2-17) as reference standards for Naloxone inhibition. Tested for inhibition were the conjugates made in Example 6 above. Results are tabulated in Table 1 and demonstrate that three of the four conjugates showed Naloxone inhibition comparable to the parent dynorphin.

ENTRY	COMPOUND	% INHIBITION AT		
		0.1 nM	10 nM	1000 nM
1	HAS	-0.6	8.04	-8.05
2	Dynorphin A 1-13-NH ₂	2.76	9.89	91.78
3	Dynorphin A 2-13-NH ₂	1.66	-0.19	44.07
4	Dynorphin A 2-17-NH ₂	-3.21	4.87	12.69

5	CCI-E (20% HSA•Dyn A 1-13(MPA)-NH ₂)	-0.76	8.27	93.66
6	CCI-F (20% HSA•Dyn A 2-13(AEA ₃ -MPA)-NH ₂)	9.11	1.40	45.15
7	CCI-G (20% HAS•Dyn A 1-13(AEA ₃ -MPA)-NH ₂)	3.72	5.37	86.35
8	CCI-H (20% HAS•MPA-AEA ₃ -Dyn A 2-17-NH ₂)	3.23	1.28	-4.06

The only conjugate which did not show inhibition was CCI-H. This conjugate (and its predecessor derivative prepared in Example 5) differs from the others in that the conjugation was produced by derivatization of the amino terminus of the dynorphin rather than of the carboxyl terminus.

In addition, CCI-H was formed from a des-Tyr dynorphin derivative, Dyn A 2-17.

The fact that conjugates CCI-E and -G functioned as well as Dyn A 1-13-NH₂ in this assay is surprising. Equally effective was CCI-F, inhibiting similarly to Dyn A 2-13-NH₂. These data suggest that the dynorphin conjugates are equipotent to the native dynorphin peptides.

Example 8

In Vivo Experiments

The following assay was conducted to show antinociceptive activity of dynorphin-albumin conjugates prepared *in vivo* and *ex vivo* with 20% HSA in mice.

The materials tested were:

Group A: Morphine (10 μ mol/kg or 3 mg/kg), 20% HSA (1 dose, i.e. 250 μ L), 0.9% saline.

Group B: Dynorphin A 1-13-NH₂ salt (10 μ mol/kg or 20 mg/kg), CCI-1017 (10 μ mol/kg or 696 mg/kg) as *ex-vivo* conjugate, and CCI-1008 (30 μ mol/kg or 70 mg/kg) as *in-vivo* conjugate.

Group C: Dynorphin A 2-13-NH₂ salt (10 μ mol/kg or 18 mg/kg), CCI-1018 (10 μ mol/kg or 697 mg/kg) as *ex-vivo* conjugate, and CCI-1010 (30 μ mol/kg or 77 mg/kg) as *in-vivo* conjugate.

5 Group D: CCI-1019 (10 μ mol/kg or 699 mg/kg) as *ex-vivo* conjugate, and CCI-1009 (30 μ mol/kg or 79 mg/kg) as *in-vivo* conjugate.

Group E: Dynorphin A 2-17-NH₂ salt (10 μ mol/kg or 25 mg/kg), CCI-1020 (10 μ mol/kg or 70 mg/kg) as *ex-vivo* conjugate, and CCI-1011 (30 μ mol/kg or 90 mg/kg) as *in-vivo* conjugate.

10 Each treatment group consisted of four time points (5 min, 1 hour, 3 hours, and 24 hours) with three male mice/dose/time point.

Experimental procedure:

15 Writhing assay (Hooke, L. P.; Lee, N. M. *J. Pharmacol. Exp. Ther.* **1995**, 273, 802-807 and Hayashi, G.; Takemori, A. E. *Eur. J. Pharmacol.* **1971**, 16, 63-66)

Approximately 1 h before the writhing assay, the mice are placed individually in transparent observation chambers for an adaptation period.

20 The number of abdominal stretches (writhes) are counted for a period of 6 min: this will be the baseline response for the assay.

Test substance (250, μ L volumes) is injected as bolus via the tail vein. At the given time periods (5 min, 1 hour, 3 hours, 24 hours) after injection of test material, the mice are injected i.p. with 2 mg/kg acetic acid (HOAc)

25

Five minutes after HOAc administration, mice are placed into transparent cylinders and the number of abdominal stretches (writhes) are counted for a period of 6 min. The average of stretches are compared to that of control (0.9% saline) group. Antinociception activity is expressed as % inhibition of the average writhes in the control group (typically 18-25).

30

WHAT IS CLAIMED IS:

1. A derivative of an antinociceptive agent, said derivative containing a reactive functional group which reacts with blood components to form stable covalent bonds, the antinociceptive agent being other than an opioid having the sequence
YdAGSFLTPRRASLGC.
2. A derivative of an antinociceptive agent according to claim 1 which reacts with blood proteins to form stable covalent bonds.
3. A derivative of an antinociceptive agent according to claim 1 which reacts with albumin to form a stable covalent bond.
4. A derivative of an antinociceptive agent according to claim 1 in which the reactive functional group is capable of reacting with an amino group, a carboxyl, or a thiol group on the blood component.
5. A derivative of an antinociceptive agent according to claim 4 in which the reactive functional group is capable of reacting with a thiol group on the blood component.
6. A derivative of an antinociceptive agent according to claim 1 prepared by reaction of an antinociceptive agent with a succinimide or maleimide compound.
7. A derivative according to claim 6 prepared by reaction of an opioid or analog thereof with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, maleimide-benzoyl-succinimide, gamma-maleimide-butyrylsuccinimide or maleimidopropionic acid.

8. A derivative of an antinociceptive agent according to claim 6 prepared by reaction of an antinociceptive agent with a maleimide compound.

5 9. A derivative of an antinociceptive agent according to claim 1 wherein the antinociceptive agent is an opioid or an analog of an opioid.

10 10. An opioid derivative according to claim 9 in which the opioid is selected from dynorphins and analogs thereof, said derivative being reactive with blood proteins.

11. An opioid derivative according to claim 9, said derivative being reactive with a thiol group on a blood protein.

15

12. A conjugate comprising a derivative of an antinociceptive agent covalently bonded to a blood component, the derivative being a derivative of an antinociceptive agent other than an opioid having the sequence YdAGSFLTPRRASLGC.

20

13. A conjugate according to claim 12 in which the blood component is a blood protein.

25 14. A conjugate according to claim 13 in which the blood protein is albumin.

30 15. A conjugate according to claim 12 in which the covalent bond is formed between an amino, carboxyl or thiol group on the blood component and a reactive functional group contained in the opioid derivative.

16. A conjugate according to claim 15 in which the covalent bond is formed between a thiol group on the blood component and a reactive functional group contained in the opioid derivative.

5 17. A conjugate according to claim 9 in which the antinociceptive agent is an opioid or an analog of an opioid.

10 18. A conjugate according to claim 17 in which the opioid derivative is a derivative of a dynorphin or an analog of a dynorphin and the blood component is a protein.

19. A conjugate according to claim 18 in which the blood component is albumin.

15 20. A blood composition comprising conjugates of one or more blood components covalently bonded to a derivative of an antinociceptive agent, said conjugates resulting from the addition of said derivative to blood.

20 21. A blood composition according to claim 20 in which the derivative is a derivative of an opioid other than an opioid having the sequence YdAGSFLTPRRASLGC.

25 22. A blood composition according to claim 21 in which the opioid is a dynorphin or an analog of a dynorphin.

30 23. A blood composition according to claim 22 in which the opioid derivative is a derivative of a dynorphin or a dynorphin analog and the blood component comprises one or more blood proteins.

24. A blood composition according to claim 20 in which the opioid derivative is covalently bonded to one or more blood proteins.

25. A blood composition according to claim 20 in which the conjugates comprise conjugates of an opioid derivative and albumin.

5 26. A blood composition according to claim 20 in which the covalent bond is formed between an amino, carboxyl or thiol group on the blood component and a reactive functional group contained in the opioid derivative.

10 27. A conjugate according to claim 20 in which the covalent bond is formed between a thiol group on the blood component and a reactive functional group contained in the derivative.

15 28. A conjugate according to claim 16 in which the blood component comprises albumin.

20 29. A derivative or a conjugate according to any of claims 1 to 28 in which the antinociceptive agent is selected from dynorphins, endorphins, deltorphins, enkephalins, and analogs of these.

 30. A derivative or a conjugate according to any of claims 1 to 29 in which the antinociceptive agent is selected from dynorphins and analogs thereof.

25 31. A derivative or a conjugate according to any of claims 1 to 29 in which the antinociceptive agent is selected from endorphins and analogs thereof.

30 32. A derivative or a conjugate according to any of claims 1 to 29 in which the antinociceptive agent is selected from deltorphins and analogs thereof.

33. A derivative or a conjugate according to any of claims 1 to 29 in which the opioid is selected from enkephalins and analogs thereof.

5 34. A method of extending the lifetime of an antinociceptive agent, said method comprising administering to the vascular system of a mammalian host a derivative of said antinociceptive agent according to any of claims 1 to 8 or 23 to 28, whereby said derivative reacts with one or more blood components so as to
10 covalently bond therewith to form a conjugate.

35. A method according to claim 34 in which the antinociceptive agent is an opioid or an analog of an opioid.

15 36. A method according to claim 35 in which the antinociceptive agent is selected from dynorphins, endorphins, deltorphins, enkephalins and analogs thereof.

20 37. A method according to claim 36 in which the opioid is selected from dynorphins and dynorphin analogs.

25 38. A method for treating a patient to alleviate pain or provide an analgesic effect comprising administering to the patient a derivative or conjugate according to any of claims 1 to 33 in a quantity effective to alleviate pain or produce an analgesic effect.

30 39. A method according to claim 34 in which a derivative according to any of claims 1 to 11 or 28 to 33 is administered to the patient and the derivative reacts with a component of the patient's blood to produce a conjugate according to any of claims 12 to 19 or 28 to 33.

40. A method according to claim 34 in which at least a portion of the patient's blood is withdrawn from the patient's body and contacted with a derivative according to any of claims 1 to 11 or 28 to 33 to form a conjugate according to any of claims 12 to 19 or 28 or 33, or a blood composition according to any of claims 20 to 23.

41. A method according to claim 34 in which a conjugate is administered to the patient.

10

42. A method according to claim 34 in which a blood component is contacted with a derivative according to any of claims 1 to 11 or 28 to 33 to form a conjugate according to any of claims 12 to 19 or 28 or 33.

15

43. A method according to claim 43 where the commercial blood component is albumin.

44. A method for removing from or controlling within a patient's blood a conjugate according to any of claims 12 to 19 or 28 to 33, said method comprising contacting the blood or the conjugate with an antibody specific for said conjugate or for the antinociceptive agent present as a component of said conjugate.

20

SEQUENCE LISTING

<110> MILNER, PETER
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BRIDON, DOMINIQUE
HOLMES, DARREN

<120> NOVEL CONJUGATES OF OPIOIDS AND ENDOGENOUS CARRIERS

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<150> US 60/064,705

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Artificial Sequence

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15



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US98/23704 (22) International Filing Date: 6 November 1998 (06.11.98) (30) Priority Data: 60/064,705 7 November 1997 (07.11.97) US (71) Applicant (for all designated States except US): CON- JUCHEM, INC. [CA/CA]; 3rd floor, Suite 3950, 225 President Kennedy Avenue West, Montreal, Quebec H2X 3Y8 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): EZRIN, Alan, M. [US/US]; 110 Quintas Lane, Moraga, CA 94556 (US). BRIDON, Dominique, P. [FR/CA]; 243 Chemin Cote Ste-Catherine, Outremont, Quebec H2V 2B2 (CA). HOLMES, Darren, L. [US/CA]; 3450 Drummond Street, Montreal, Quebec H3G 1T3 (CA). MILNER, Peter [GB/US]; 14690 Manuela Road, Los Altos Hills, CA 94022 (US). (74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111-4262 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 19 August 1999 (19.08.99)
(54) Title: NOVEL CONJUGATES OF OPIOIDS AND ENDOGENOUS CARRIERS		
(57) Abstract <p>Conjugates are prepared from antinociceptive agents, particularly opioids or opioid analogs, more particularly dynorphins, endorphins, deltorphins, enkephalins or analogs thereof, by combining said antinociceptive agent with a material providing a functionally reactive group capable of reacting with a blood component (preferably a blood cell or protein). Said conjugates permit extension of the therapeutic life of the antinociceptive agent. They may be administered to patients to alleviate pain, produce analgesic effects, or assist in cases of narcotics withdrawal, and may also be used as probes for receptor activity. The administration to the patient may be made either <i>in vivo</i> or <i>ex vivo</i> and may be performed by either introducing the derivative including the reactive functional group into the patient's vascular system or preparing such a conjugate externally (or <i>in vitro</i>) and introducing that conjugate to the patient's vascular system.</p>		

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EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/23704

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/665 C07K14/70 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 08220 A (G HOUEN & A HOLM) 13 June 1991 see page 23, line 18 ---	1-6, 13-18, 22-44
X	CHEMICAL ABSTRACTS, vol. 106, no. 17, 27 April 1987 Columbus, Ohio, US; abstract no. 136622, G E ISOM: "Production and characterization of anti-morphine anti-idiotypic antibodies" XP002106421 & METHODOL. SURV. BIOCHEM. ANAL., vol. 15, 1985, pages 109-114, see abstract --- -/--	1-6, 13-18, 22-44



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 June 1999

Date of mailing of the international search report

06/07/1999

Name and mailing address of the ISA

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Authorized officer

Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/23704

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 119, no. 19, 8 November 1993 Columbus, Ohio, US; abstract no. 195892, J A D ANAND & A OOMMEN: "The rat brain delta opioid receptor studied with anti-idiotypic antibodies to anti-leucin enkephalin" XP002106422 & INDIAN J. BIOCHEM. BIOPHYS., vol. 30, no. 2, 1993, pages 117-122, see abstract	1-6, 13-18, 22-44
X	--- CHEMICAL ABSTRACTS, vol. 102, no. 3, 21 January 1985 Columbus, Ohio, US; abstract no. 22715, XP002106423 & JP 59 138958 A (MITSUBISHI CHEMICAL INDUSTRIES CO. LTD.) 9 August 1984 see abstract	1-6, 13-18, 22-44
X	--- CHEMICAL ABSTRACTS, vol. 101, no. 15, 8 October 1984 Columbus, Ohio, US; abstract no. 123197, A C CUELLO ET AL.: "Characterization and immunocytochemical application of monoclonal antibodies against enkephalins " XP002106424 & J. HISTOCHEM. CYTOCHEM. , vol. 32, no. 9, 1984, pages 945-957, see abstract	1-6, 13-18, 22-44
X	--- CHEMICAL ABSTRACTS, vol. 94, no. 21, 25 May 1981 Columbus, Ohio, US; abstract no. 171603, W B WATKINS ET AL.: "Presence of beta-endorphin-like immunoreactivity in the anterior pituitary gland of rat and man and evidence for the differential localization with ACTH " XP002106425 & CELL TISSUE RES., vol. 215, no. 3, 1981, pages 577-589, see abstract --- -/--	1-6, 13-18, 22-44

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/23704

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 91, no. 19, 5 November 1979 Columbus, Ohio, US; abstract no. 153781, J BORVENDEG ET AL.: "Radioimmunoassay of beta-endorphin: immunoreactive substances in the brain and pituitary" XP002106426 & INT. CONGR. SER. EXCERPTA MEDICA, vol. 471 (Endorphins '78), 1978, pages 177-186, see abstract ---	1-6, 13-18, 22-44
X	CHEMICAL ABSTRACTS, vol. 86, no. 7, 14 February 1977 Columbus, Ohio, US; abstract no. 39691, B A WEISSMANN ET AL.: "Specific antiserum to Leu-enkephalin and its use in a radioimmunoassay" XP002106427 & FEBS LETTERS., vol. 70, no. 1, 1976, pages 245-248, AMSTERDAM NL see abstract ---	1-6, 13-18, 22-44
A	B L KIEFFER ET AL.: "32P-Labeled opioid peptides with high affinity for the delta-opioid receptor" ANALYTICAL BIOCHEMISTRY., vol. 215, no. 1, 1993, pages 1-8, XP002106420 NEW YORK US cited in the application see the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/23704

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 34-44 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-44
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
As the very obscure wording of the claims (see e.g. the expressions "blood proteins" or "reactive functional group") does not permit a complete search for economical reasons, the search was limited to the opioid agents of claim 28.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/23704

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9108220 A	13-06-1991	AU 6892991 A	26-06-1991
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